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ORIGINAL ARTICLE

Trajectories of glycaemic traits exhibit sex-specific associations with hepatic iron and fat content: Results from the KORA-MRI study

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) represents a major disease burden in the population. While the bidirectional association between NAFLD and diabetes is established, little is known about the association of hepatic iron content and glycaemia. Moreover, analyses of sex-specific effects and of dynamic changes in glycaemia are scarce.

Methods: We investigated 7-year sex-specific trajectories of glycaemia and related traits (HbA1c, fasting glucose, fasting insulin, HOMA-IR, 2-h glucose and cross-sectional 2-h insulin) in a sample from a population-based cohort ($N = 365$; 41.1% female). Hepatic iron and fat content were assessed by 3T-Magnetic Resonance Imaging (MRI). Two-step multi-level models adjusted for glucose-lowering medication and confounders were applied.

Results: In women and men, markers of glucose metabolism correlated with hepatic iron and fat content. Deterioration of glycaemia was associated with increased hepatic

Abbreviations: BMI, Body Mass Index; CI, confidence interval; CVD, cardiovascular disease; HbA1c, Haemoglobin A1c; HOMA-IR, homeostasis model of insulin resistance; KORA, Kooperative Gesundheitsforschung in der Region Augsburg; MRI, magnetic resonance imaging; NAFLD, non-alcoholic fatty liver disease; OGTT, oral glucose tolerance test; T2D, type 2 diabetes.

iron content in men (normoglycaemia to prediabetes: beta = 2.21 s^{-1}, 95% CI [0.47, 3.95]). Additionally, deterioration of glycaemia (e.g. prediabetes to diabetes: 1.27 log(%), [0.84, 1.70]) and trajectories of glucose, insulin and HOMA-IR were significantly associated with hepatic fat content in men. Similarly, deterioration of glycaemia as well as trajectories of glucose, insulin and HOMA-IR was significantly associated with increased hepatic fat content in women (e.g. trajectory of fasting insulin: 0.63 log(%), [0.36, 0.90]).

Conclusions: Unfavourable 7-year trajectories of markers of glucose metabolism are associated with increased hepatic fat content, particularly in women, whereas the association with hepatic iron content was less clear. Monitoring changes of glycaemia in the sub-diabetic range might enable early identification of hepatic iron overload and steatosis.

KEYWORDS
diabetes, glucose, HbA1c, hepatic fat, hepatic iron, insulin, NAFLD, sex, trajectories

1 | BACKGROUND

Non-alcoholic fatty liver disease (NAFLD), defined as an excessive build-up of hepatic fat irrespective of alcohol consumption, viral infection, or other causes, is a common and rapidly rising liver disease worldwide. NAFLD can progress to steatohepatitis, fibrosis, cirrhosis and hepatocellular carcinoma, thus representing a precursor state of potentially serious and life-threatening liver outcomes.[1]

NAFLD is tightly connected to insulin resistance, which causes impaired lipolysis and excess fatty acid transport to the hepatocytes, while dysregulated adipose tissue lipolysis in turn promotes insulin resistance. Thus, Type 2 diabetes (T2D) and NAFLD frequently co-exist.[2] Recent meta-analyses estimated that 55.5% of individuals with T2D also had NAFLD[3] and that NAFLD doubles the risk of incident T2D.[4] In addition, Mendelian randomization studies have demonstrated a causal, bi-directional relationship.[5]

Diabetes has also been identified as a risk factor of progression to liver cirrhosis and carcinoma.[6] However, although NAFLD development and progression are known to differ according to biological sex,[7] sex-specific impacts of diabetes on hepatic fat accumulation have not been comprehensively studied. Moreover, since glucose metabolism is dynamic, the development of glycaemia over time might also affect hepatic fat content.

Recently, both animal and human studies have implicated hepatic iron content as a potential factor driving the progression from NAFLD to steatohepatitis via ferroptosis-induced inflammation and necrosis.[8,9] An association between markers of iron metabolism and liver disease has already been established.[10] A recent Mendelian randomization study using data from the UK Biobank showed tentative evidence for a potential causal association of liver iron on fatty liver.[11]

At the same time, oxidative stress induced by iron overload leads to increased insulin resistance, establishing a link between circulating markers of iron metabolism and T2D.[12] Sex-specific associations, with higher T2D risk conferred by elevated ferritin levels in women compared to men, have been reported.[13,14] However, the link between hepatic iron as a major storage site of body iron and glucose metabolism is less clear and again there is a lack of data regarding sex-specific effects.

Quantitative data on hepatic iron and hepatic fat infiltration in population-based studies are scarce. Most non-clinical studies define NAFLD based on ultrasound, which is an established validated technique but cannot estimate hepatic iron content and does not precisely quantify hepatic fat content. However, given the societal burden of NAFLD in the general population, it is crucial to study risk factors and implications in a population-based setting. Magnetic resonance imaging (MRI) is a non-invasive, radiation-free, albeit costly modality to accurately assess hepatic iron and fat content.[15]

With the present analysis, we aim to tackle some of the currently open questions. In a sample from a population-based cohort, we analyse the association of changes in glycaemia over time, as well as the longitudinal trajectories of markers of glucose metabolism, with MRI-derived hepatic iron and hepatic fat content separately for men and women.

Key points

Diabetes mellitus and non-alcoholic fatty liver disease frequently co-exist. We show that not only cross-sectional values but also the deterioration of glycaemic traits over time are associated with higher values of hepatic fat content in a sex-specific fashion. Moreover, our findings demonstrate that trajectories of glucose metabolism are related to hepatic iron content in men.

2 | METHODS

2.1 | Study sample

We used data from two examination time points of a longitudinal, population based cohort study from Southern Germany. Details of the general setup of the Cooperative Health Research in the Region of Augsburg (KORA) studies have been described elsewhere.[16] Our sample is based on $N = 400$ individuals that underwent whole-body MRI during the examination in 2013–2014 (KORA-FF4, total $N = 2279$, defined as Exam 2 in the present paper). The MRI sub-study aimed to evaluate subclinical cardiometabolic disease burden in individuals with impaired glucose metabolism. Individuals with prevalent cardiovascular disease (stroke, myocardial infarction, revascularization) or any contraindications to MRI were excluded.[17] For these $N = 400$ individuals, we used clinical data that was assessed during the examination 7 years prior in 2006–2007 (KORA F4, total $N = 3080$, defined as Exam 1 in the present paper).

All KORA studies are approved by the ethics committee of the Bavarian Chamber of Physicians, and the MRI sub-study was additionally approved by the ethics committee of the Ludwig-Maximilians-University Munich. The study complies with the Declaration of Helsinki, including written informed consent from all participants. All participants underwent a standardized face-to-face interview, a blood draw and a comprehensive physical examination conducted by trained examiners at both examination time points.

2.2 | Outcome assessment

Participants underwent whole-body MRI performed on a 3 Tesla MRI scanner (Magnetom Skyra; Siemens AGA, Siemens Healthineers, Erlangen, Germany). Hepatic iron and fat content were obtained in the left and right liver lobes using the high-speed T2-corrected multi-echo sequence (HISTO).[18] Iron was measured as relaxation rate R2* in s^{-1}, and fat content was measured as mean proton density fat fraction in percent.[19] For statistical analysis, we used the arithmetic mean of left and right liver lobe as outcome. Mild hepatic iron overload was defined as $R2^* > 41\,s^{-1}$.[15] Hepatic steatosis was defined as proton density fat fraction >6.4%.[20] Hepatic outcomes were available only at Exam 2.

2.3 | Exposure assessment

Markers of glucose metabolism included diabetes status, Haemoglobin A1c (HbA1c), fasting glucose, fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR), 2-h glucose and 2-h insulin. Glycaemia (normoglycaemia, prediabetes, diabetes) was categorized based on prior physician diagnosis, or an Oral Glucose Tolerance Test (OGTT) conducted during the study examination in persons without previous clinically diagnosed diabetes. According to World Health Organization criteria, normoglycaemia was defined as fasting blood glucose concentration below 110 mg/dL and 2-h glucose below 140 mg/dL. Impaired fasting glucose (fasting glucose concentration between 110 and 125 mg/dL) and impaired glucose tolerance (2-h glucose between 140 mg/dL and 200 mg/dL) were subsumed as prediabetes. Diabetes was newly diagnosed when fasting glucose concentrations exceeded 125 mg/dL and/or 2-h glucose concentrations exceeded 200 mg/dL. HbA1c was measured by a turbidimetric inhibition immunoassay at Exam 1 and by a cation-exchange high-performance liquid chromatographic assay at Exam 2. HOMA-IR was calculated as (fasting insulin (mU/L) × fasting glucose (mmol/L))/22.5. Glucose-lowering medication comprised ATC codes A10.

Availability of measurements varied between exams and individuals (Figure 1). Since OGTT was only performed in individuals without prior known diabetes, 2-h glucose and insulin measurements were not available for these participants. Moreover, 2-h insulin was only measured in Exam 2. HOMA-IR was only calculated for individuals who did not use glucose-lowering medication.

2.4 | Risk factors assessment

Body height and weight were measured by Seca's measuring system (Seca GmbH&Co, KG) with accuracy of up to 0.1 cm and 0.1 kg, respectively. Body-Mass-Index (BMI) was calculated as weight divided by squared height (kg/m^2). Waist circumference was measured at the level midway between the lower ribs margin and the iliac crest.

Total cholesterol was measured by enzymatic colorimetric assay.[21]

Blood pressure was measured with an OMRON type HEM-705CP oscillometric device three times (3 min intervals) after participants had rested in a seated position for at least five minutes. The mean of the 2nd and 3rd measurements was used as the final value. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or intake of antihypertensive medication under the awareness of having hypertension.

Medication intake, alcohol consumption and menopausal status were self-reported in the medical interview. Women were categorized into pre- and post-menopausal, as described in Maier et al.[21]

Genotypes for selected SNPs previously reported to be associated with either hepatic fat content or iron markers, including variants in *HFE* and *PNPLA3*, were obtained with Affymetrix Axiom Chip and subsequently imputed with HRC panel 1.1.[21]

2.5 | Statistical analysis

All analyses were stratified by sex. Continuous variables were described as arithmetic mean and standard deviation for each exam. Categorical variables were described as counts and percentages. Changes between exams were quantified by paired *t*-test and Cochran's Q test.

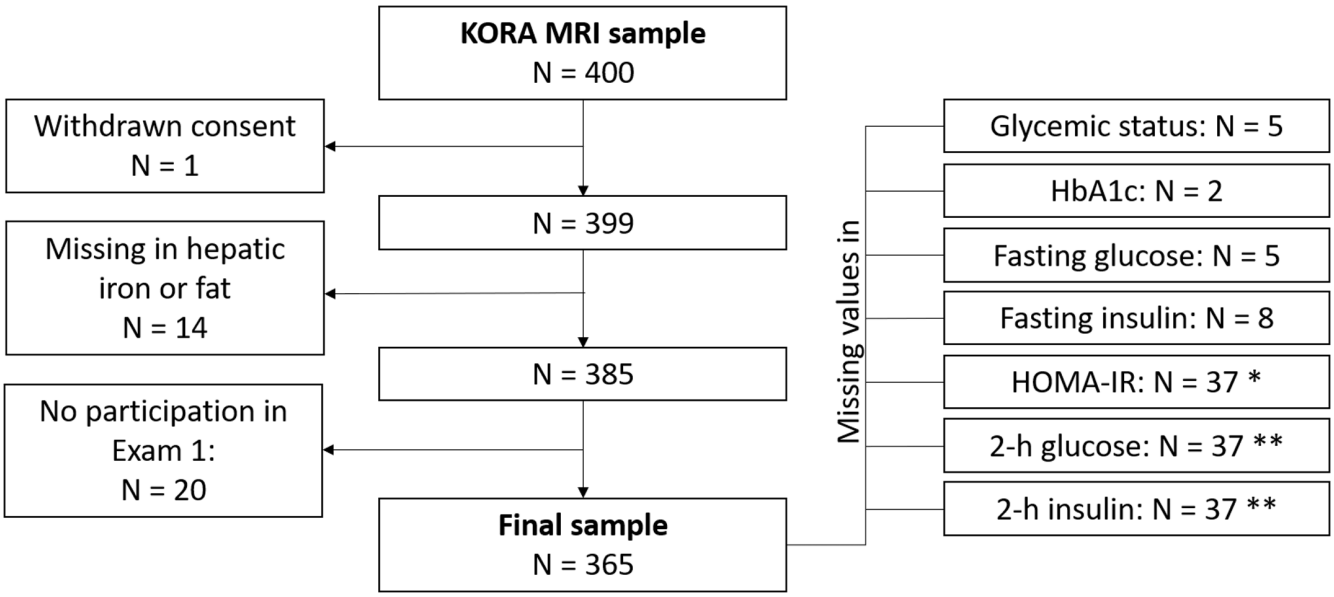

FIGURE 1 Flowchart of the KORA MRI study sample.

Trajectories of markers of glucose metabolism across exams were visualized by line plots. Correlations between markers of glucose metabolism and hepatic iron or fat content were visualized by scatter plots and quantified by Spearman's Rho correlation coefficient and corresponding *p*-value. Distribution of hepatic iron and fat content according to change in diabetes status between both exams were visualized by boxplots and quantified by *t*-test. Variation of hepatic iron and fat content according to genotypes at selected SNPs were visualized by boxplots for the whole sample.

To assess the association of changes in glycaemia between exams with outcomes hepatic iron and fat content, we used a linear regression model adjusted for (1) age at Exam 1, (2) age, BMI and alcohol consumption at Exam 1. Individuals with sustained normoglycaemia served as the reference group. As sensitivity analyses, models were adjusted for WC instead of BMI and for women, models were additionally adjusted for menopausal status. To assess the association of trajectories of glucose metabolism markers between exams with the outcomes, we used a two-step multilevel model.[22] In step one, individual trajectories of every marker were calculated by a linear mixed model with random slope, representing estimated individual variations from the population rate of change for each marker. In this model, also changes in use of glucose-lowering medication were included, where applicable. In step two, the recorded trajectories from step one were standardized and entered into a linear model, with the adjustments outlined above plus the standardized value of the respective marker at Exam 1. For the marker 2-h insulin that was only available at Exam 2, a linear regression model with the adjustments outlined above was used.

For all regression models, the outcome hepatic fat content was log-transformed due to skewness. Results were reported as beta coefficients with corresponding 95% confidence intervals (CI) and *p*-value. *p*-values less than .05 were considered to denote statistical significance.

Statistical analyses were performed using R version 3.6.1.

3 | RESULTS

3.1 | Study sample

Of the original 400 participants of the KORA-MRI study, one individual was excluded because they retroactively withdrew the consent for data usage. Further 14 individuals were excluded because of missing MRI data due to imaging artefacts or insufficient image quality. Further 20 individuals were excluded because they did not participate in Exam 1 (Figure 1). Thus, the final sample consisted of $N = 365$ individuals, thereof 215 (58.9%) men and 150 (41.1%) women.

Mean age at Exam 2 was 56.3 years (standard deviation (SD) 9.4 years) for men and 56.6 years (9.1 years) for women (Table 1). Among cardiometabolic risk factors, waist circumference increased significantly between the exams in both sexes, whereas BMI, blood pressure and cholesterol levels did not increase significantly. In men, mean hepatic iron content was 41.8 s^{-1} (4.9 s^{-1}) and mean hepatic fat content 10.7% (8.9%) at Exam 2, while n = 83 (38.6%) had both hepatic iron overload and steatosis. In women, mean iron content was 39.1 s^{-1} (4.0 s^{-1}) and mean fat content was 6.5% (6.3%), while $n = 22$ (14.7%) had both iron overload and steatosis, respectively. Hepatic iron and fat content varied according to genotype at selected SNPs (Figures S1 and S2).

TABLE 1 Characteristics of the participants at both exams.

	Men			Women		
	Exam 1	Exam 2	*p*-value	Exam 1	Exam 2	*p*-value
n	215	215		150	150	
Risk factors						
Age [years]	49.3 (9.4)	56.3 (9.4)		49.6 (9.1)	56.6 (9.1)	
BMI [kg/m^2]	27.7 (4.0)	28.4 (4.5)	.086	27.0 (5.0)	27.7 (5.5)	.255
Waist circumference [cm]	98.9 (11.0)	103.4 (12.6)	<.001	87.1 (13.0)	92.0 (14.1)	.002
Total cholesterol [mg/dL]	213.6 (36.3)	217.6 (37.8)	.264	217.2 (38.7)	218.9 (34.4)	.687
HDL cholesterol [mg/dL]	48.7 (11.3)	56.0 (15.1)	<.001	61.2 (13.9)	70.9 (17.5)	<.001
LDL cholesterol [mg/dL]	139.2 (31.6)	142.2 (33.6)	.342	135.5 (36.3)	135.9 (32.0)	.919
Triglycerides [mg/dL]	147.5 (101.2)	152.5 (101.5)	.608	96.9 (55.7)	102.3 (46.7)	.365
Systolic blood pressure [mmHg]	125.6 (15.2)	125.9 (16.2)	.868	114.7 (15.6)	113.6 (14.6)	.539
Diastolic blood pressure [mmHg]	78.4 (9.3)	77.7 (10.5)	.464	73.9 (9.0)	72.3 (8.5)	.11
Hypertension	61 (28.4%)	82 (38.1%)	.041	34 (22.7%)	44 (29.3%)	.236
Alcohol consumption [g/day]	25.4 (27.4)	25.9 (26.9)	.835	8.2 (13.0)	8.5 (14.3)	.839
Postmenopausal				54 (36.0%)	88 (58.7%)	<.001
Medication						
Antihypertensive	29 (13.5%)	51 (23.7%)	.009	26 (17.3%)	42 (28.0%)	.039
Lipid-lowering	19 (8.8%)	21 (9.8%)	.868	7 (4.7%)	17 (11.3%)	.055
Glucose-lowering	7 (3.3%)	18 (8.4%)	.039	4 (2.7%)	12 (8.0%)	.072
Markers of glucose metabolism						
Diabetes			<.001			.246
Normoglycaemia	157 (74.1%)	119 (55.3%)		116 (78.4%)	105 (70.0%)	
Prediabetes	39 (18.4%)	59 (27.4%)		23 (15.5%)	31 (20.7%)	
Diabetes	16 (7.5%)	37 (17.2%)		9 (6.1%)	14 (9.3%)	
HbA1c [%]	5.5 (0.6)	5.6 (0.8)	.209	5.5 (0.5)	5.6 (0.6)	.043
Fasting glucose [mg/dL]	101.1 (19.3)	107.9 (25.4)	.002	93.4 (14.8)	99.7 (18.7)	.002
Fasting insulin [μU/mL]	11.2 (7.1)	12.3 (8.6)	.143	10.0 (6.5)	9.9 (5.8)	.889
HOMA-IR[a]	2.8 (2.0)	3.2 (2.5)	.093	2.4 (1.9)	2.4 (1.6)	.88
2-h glucose [mg/dL][a]	110.3 (33.9)	118.4 (45.5)	.047	106.9 (37.1)	106.5 (35.3)	.916
2-h insulin [μU/mL][a]		72.9 (79.4)			58.1 (48.1)	
MRI derived liver values						
Hepatic iron content [s^{-1}]		41.8 (4.9)			39.1 (4.0)	
Hepatic fat content [%]		10.7 (8.9)			6.5 (6.3)	
No iron overload + no steatosis		52 (24.2%)			85 (56.7%)	
Iron overload (no steatosis)		40 (18.6%)			19 (12.7%)	
Steatosis (no iron overload)		40 (18.6%)			24 (16.0%)	
Iron overload + steatosis		83 (38.6%)			22 (14.7%)	

Note: Continuous variables are described as arithmetic mean and standard deviation. Categorical variables are described as counts and percentages. Changes between exams were quantified by paired *t*-test and Cochran's Q test, respectively.

[a]Only available in participants without established diabetes, for sample sizes see Figure 1.

3.2 | Trajectories of glycaemia and of markers of glucose metabolism

Between exams, 67.4% of men and 78.3% of women maintained their glycaemia state (Table S1). Prevalence of diabetes at Exam 2 was 17.2% in men and 9.3% in women. The majority of men (51.9%) remained normoglycaemic, whereas 19.8% had incident prediabetes and 7.1% progressed from prediabetes to diabetes between exams. In women, the majority remained normoglycaemic (65.5%), whereas 12.8% had incident prediabetes and 4.1% progressed to diabetes (Table S1).

Mean fasting glucose, fasting insulin, HOMA-IR and 2-h glucose increased in men (e.g. fasting glucose: 101.1 mg/dL (19.3 mg/dL)

in Exam 1 to 107.9 mg/dL (25.4 mg/dL) in Exam 2), whereas mean trajectories of fasting insulin, HOMA-IR and 2-h glucose remained stable in women (e.g. fasting insulin 10.0 μU/ml (6.5 μU/ml) in Exam 1 to 9.9 μU/ml (5.8 μU/ml) in Exam 2). There were however substantial differences in trajectories according to glycaemia at Exam 2 (Figure 2). In particular, HOMAR-IR and fasting insulin

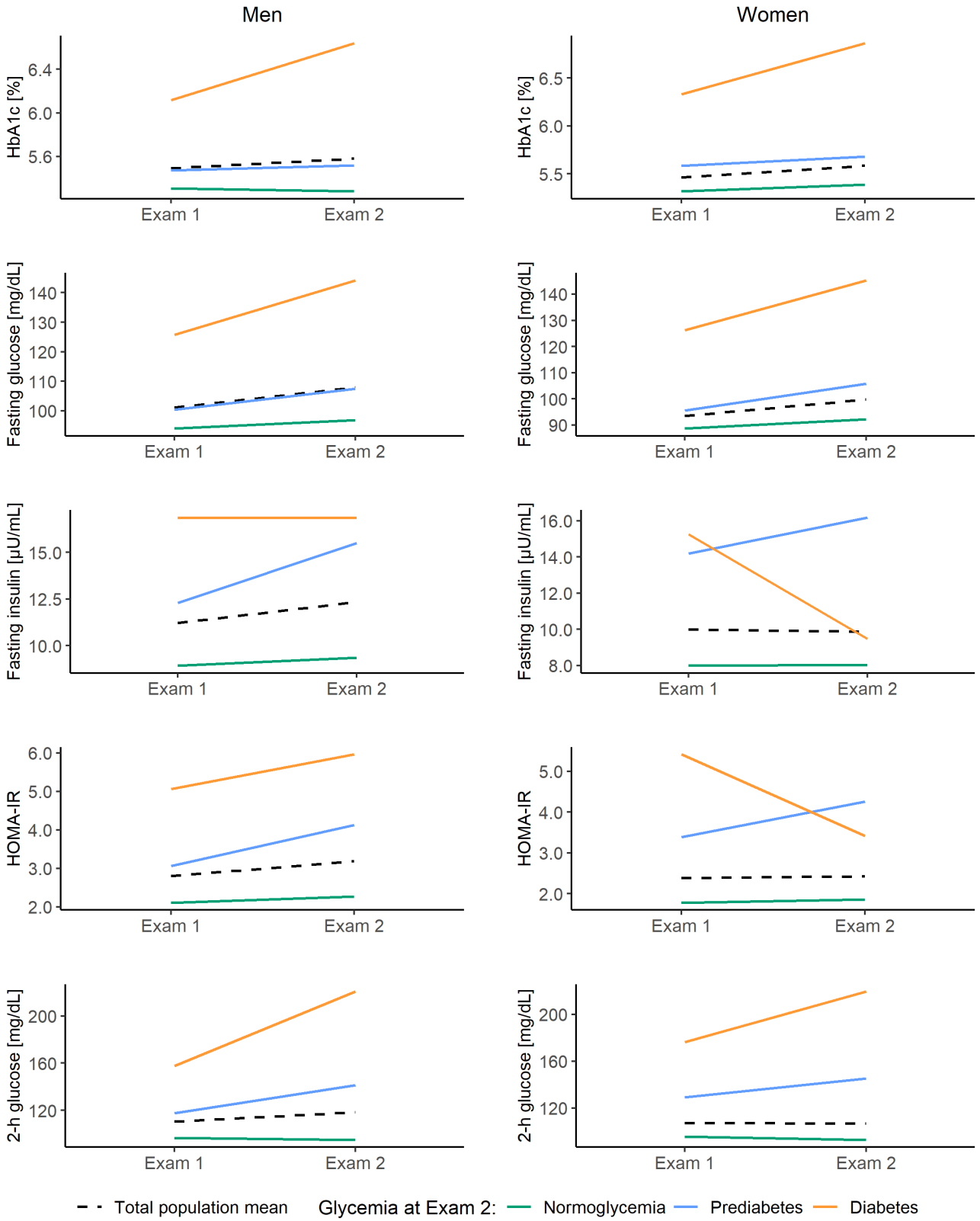

FIGURE 2 Change in mean levels of markers of glucose metabolism from Exam 1 to Exam 2 in the total population and stratified by glycaemia at Exam 2. 2-h insulin was only measured in Exam 2 and is thus not shown.

decreased in women with diabetes, which we did not observe in men.

3.3 | Correlation of glucose metabolism with hepatic iron and fat content

Markers of glucose metabolism were correlated with hepatic iron and fat content to varying degrees (Figures 3 and 4, Table S2). Generally, correlations with hepatic fat content were stronger than correlations with hepatic iron content, and correlations were stronger for glycaemic traits measured at Exam 2 (concurrent to the assessment of iron and fat content) compared to Exam 1. For both iron and fat, correlations were generally stronger in women than in men.

In men, HbA1c values at both exams were negatively correlated with hepatic iron content (R Exam 1 = −0.14, $p = .037$). Fasting glucose was not significantly correlated with iron, in contrast to 2-h glucose (R Exam 1 = 0.17, $p = .016$). Fasting and 2-h insulin and HOMA-IR were significantly correlated with hepatic iron content only for the values measured at Exam 2. All markers at both exams were significantly positively correlated with hepatic fat content, with the strongest correlation for HOMA-IR measured at Exam 2 (R Exam 2 = 0.65, $p < .001$) (Figure 3, Table S2).

In women, HbA1c values at both exams were positively correlated with hepatic iron content (R Exam 1 = 0.20, $p = .012$). Similarly, fasting glucose, fasting insulin and HOMA-IR, as well as 2-h glucose and 2-h insulin measured at Exam 2, were positively correlated with hepatic iron content. All markers at both exams were significantly positively correlated with hepatic fat content with the strongest correlation for HOMA-IR measured at Exam 2 (R Exam 2 = 0.70, $p < .001$) (Figure 4, Table S2).

3.4 | Association of changes in glycaemia with hepatic iron and fat content

At Exam 2, hepatic iron content was highest in men with prediabetes: 43.2 s^{-1} (4.4 s^{-1}), compared to men with normoglycaemia (41.2 s^{-1} (4.6 s^{-1})) and diabetes (41.6 s^{-1} (5.8 s^{-1})). Men with incident prediabetes and men with sustained prediabetes had comparable values of hepatic iron content. In contrast, men who progressed from prediabetes to diabetes had higher values compared to men with sustained diabetes (43.0 s^{-1} (7.5 s^{-1}) vs. 40.6 s^{-1} (4.3 s^{-1}), Figure 5). Compared to men with stable normoglycaemia, incident prediabetes was associated with an increase of 2.21 s^{-1} (CI [0.47, 3.95]) in hepatic iron content after adjustment for age, BMI and alcohol consumption (Table 2).

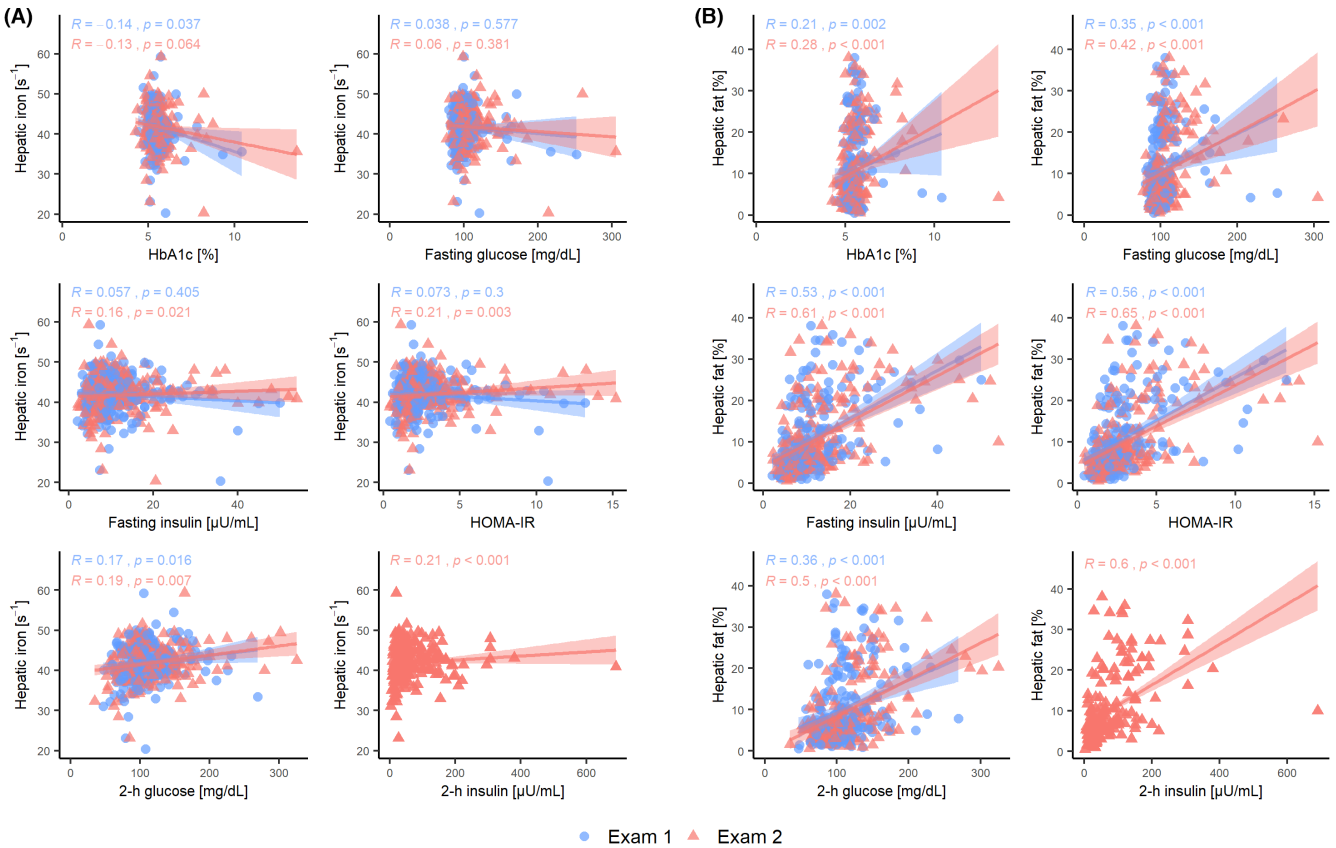

FIGURE 3 Correlation of markers of glucose metabolism with hepatic iron (A) and fat content (B) at Exam 1 and Exam 2 in men. R denotes Spearman's Rho.

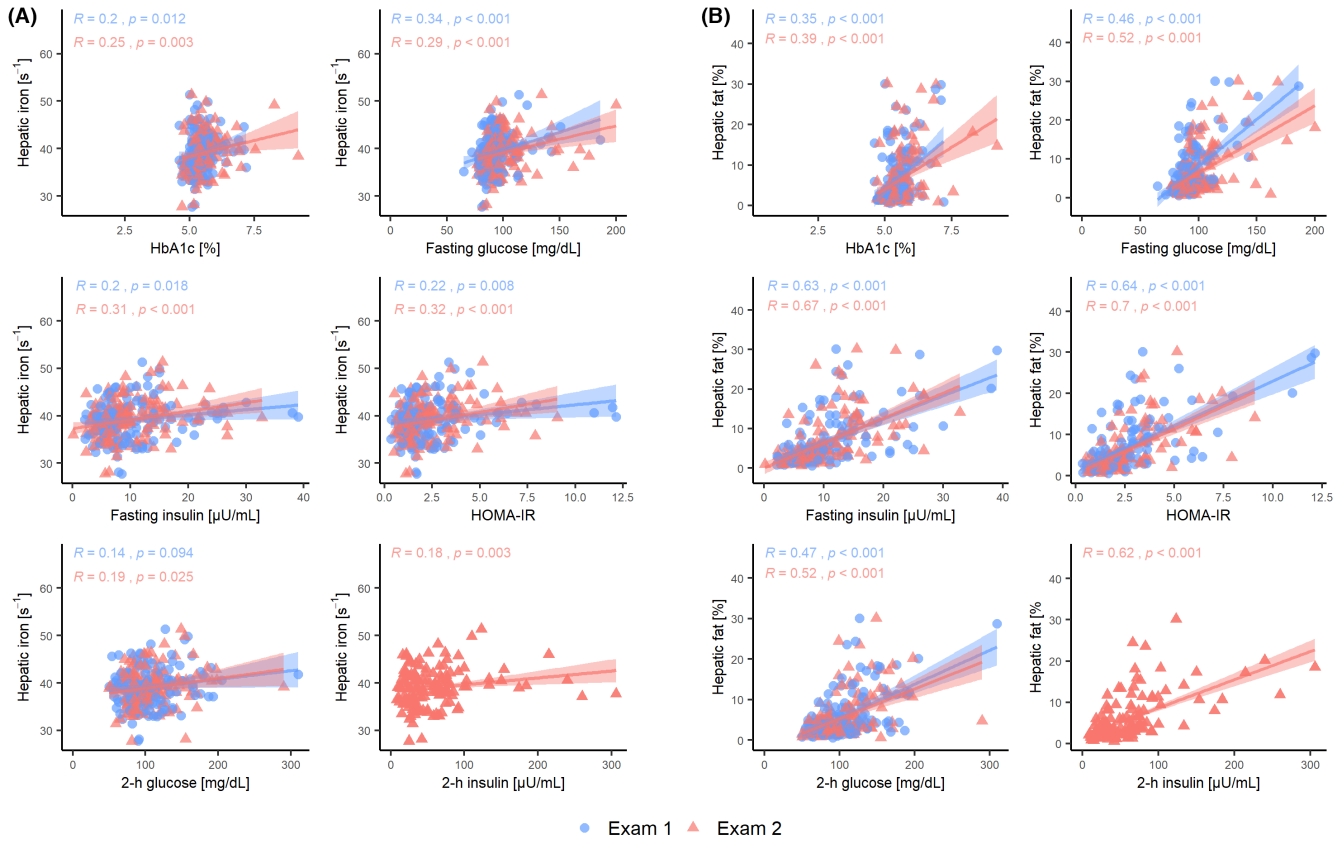

FIGURE 4 Correlation of markers of glucose metabolism with hepatic iron (A) and fat content (B) at Exam 1 and Exam 2 in women. R denotes Spearman's Rho.

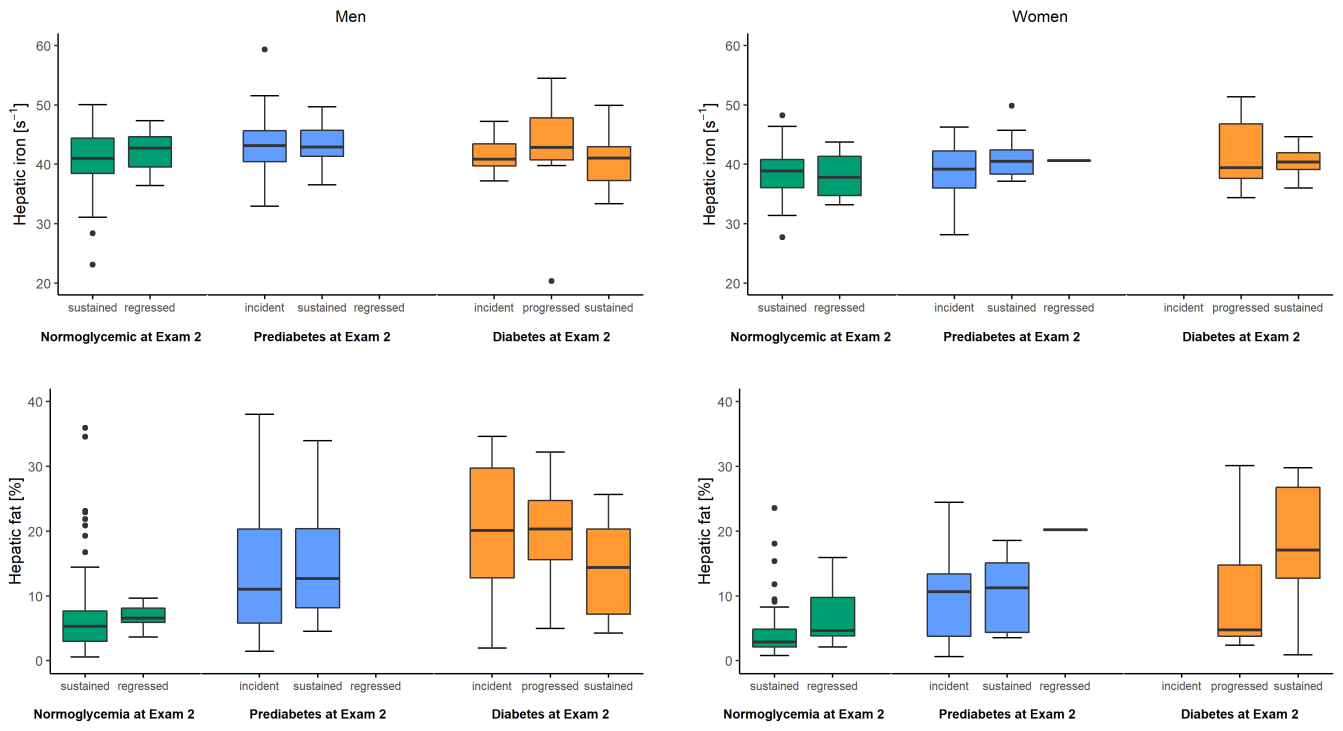

FIGURE 5 Distribution of hepatic iron and fat content according to change in glycaemia between both exams in men and women. 'Sustained': remained glycaemic in Exam 1 and Exam 2, 'Regressed': Regression from prediabetes in Exam 1 to normoglycaemia in Exam 2, or from diabetes in Exam 1 to prediabetes or normoglycaemia in Exam 2, 'Incident': normoglycaemia in Exam 1 and incident prediabetes or diabetes in Exam 2, 'progressed': prediabetes in Exam 1 and diabetes in Exam 2. Sample sizes for the respective groups are given in Table S1.

TABLE 2 Associations of changes of glycaemia with outcomes hepatic iron and fat content.

Glycaemic status (Exam 1 – Exam 2)	Adjustment	Men				Women			
		Hepatic iron content		Hepatic fat content		Hepatic iron content		Hepatic fat content	
		β (95% CI)	*p*-value	*β* (95% CI)	*p*-value	*β* (95% CI)	*p*-value	*β* (95% CI)	*p*-value
Sustained Normoglycaemia	Age	Reference		Reference		Reference		Reference	
	Age, BMI, alcohol	Reference		Reference		Reference		Reference	
Prediabetes – Normoglycaemia	Age	0.83 (−2.88, 4.54)	.659	0.28 (−0.31, 0.87)	.351	−2.33 (−5.29, 0.64)	.123	0.34 (−0.26, 0.94)	.262
	Age, BMI, alcohol	0.32 (−3.36, 4.01)	.863	0 (−0.54, 0.55)	.996	−1.96 (−4.82, 0.91)	.179	0.38 (−0.17, 0.93)	.17
Diabetes – Normoglycaemia[a]	—	—	—	—	—	—	—	—	—
Normoglycaemia – Prediabetes	Age	**2.07 (0.34, 3.80)**	**.020**	**0.74 (0.46, 1.01)**	**<.001**	−0.14 (−1.88, 1.60)	.873	**0.78 (0.43, 1.13)**	**<.001**
	Age, BMI, alcohol	**2.21 (0.47, 3.95)**	**.013**	**0.59 (0.33, 0.85)**	**<.001**	−1.21 (−3.06, 0.64)	.198	**0.37 (0.01, 0.73)**	**.041**
Sustained Prediabetes	Age	1.74 (−0.80, 4.28)	.178	**0.95 (0.55, 1.36)**	**<.001**	0.49 (−1.80, 2.78)	.672	**0.78 (0.32, 1.25)**	**.001**
	Age, BMI, alcohol	1.66 (−0.88, 4.21)	.199	**0.71 (0.34, 1.09)**	**<.001**	−0.21 (−2.51, 2.09)	.855	**0.45 (0.01, 0.89)**	**.046**
Diabetes – Prediabetes[a]	—	—	—	—	—	—	—	—	—
Normoglycaemia – Diabetes	Age	0.38 (−3.96, 4.73)	.862	**1.03 (0.34, 1.72)**	**.004**	—	—	—	—
	Age, BMI, alcohol	0.73 (−3.63, 5.09)	.742	**0.66 (0.02, 1.31)**	**.044**	—	—	—	—
Prediabetes – Diabetes	Age	1.57 (−1.11, 4.25)	.250	**1.27 (0.84, 1.70)**	**<.001**	1.65 (−1.30, 4.59)	.272	0.58 (−0.02, 1.18)	.056
	Age, BMI, alcohol	2.08 (−0.64, 4.80)	.133	**1.03 (0.63, 1.43)**	**<.001**	0.81 (−2.08, 3.69)	.58	0.47 (−0.08, 1.03)	.094
Sustained Diabetes	Age	−1.03 (−3.73, 1.66)	.450	**0.87 (0.44, 1.30)**	**<.001**	0.43 (−2.15, 3.01)	.742	**1.25 (0.73, 1.77)**	**<.001**
	Age, BMI, alcohol	−0.68 (−3.37, 2.00)	.617	**0.72 (0.32, 1.12)**	**<.001**	−0.2 (−2.80, 2.39)	.876	**0.88 (0.38, 1.38)**	**.001**

Note: Results from a linear regression model with changes in glycaemia as a categorical exposure and hepatic iron and fat content as continuous outcome. Hepatic fat content was log-transformed before analysis.

[a]No calculation possible due to too small sample.

Hepatic fat content was highest in men with diabetes: 17.4% (9.0%) as compared to men with normoglycaemia (7.0% (6.4%)) and prediabetes (14.2% (9.3%)). Men who had incident diabetes, or progressed from prediabetes to diabetes, had higher values compared to men with sustained diabetes (19.8% (8.4%) vs. 13.8% (7.3%), Figure 5). After adjustment, effect estimates were largest for progression from prediabetes to diabetes (1.03 log(%), CI (0.63, 1.43)), compared to those with sustained normoglycaemia (Table 2).

In women, hepatic iron content was highest for those with diabetes: 41.0 s^{-1} (4.7 s^{-1}) compared to women with normoglycaemia (38.7 s^{-1} (3.8 s^{-1})) and prediabetes (39.8 s^{-1} (4.3 s^{-1})). Women who progressed from prediabetes to diabetes had higher hepatic iron content values than those with sustained diabetes (41.8 s^{-1} (6.8 s^{-1}) vs. 40.4 s^{-1} (2.6 s^{-1}), Figure 5). However, after adjustment for age, BMI and alcohol, there were no significant associations of change in glycaemia with hepatic iron content (Table 2).

Hepatic fat content was also highest in women with diabetes: 14.8% (10.8%), compared to women with normoglycaemia 4.2% (3.7%) and prediabetes (10.3% (6.1%)). Women with sustained diabetes had higher values of hepatic fat than those who progressed from prediabetes to diabetes (10.6% (11.2%) vs. 17.9% (10.0%), Figure 5). Sustained diabetes had the largest effect estimate for increased hepatic fat (0.88 log(%), CI [0.38, 1.38], Table 2). Additional adjustment for menopausal status did not substantially affect the results (Table S3).

In sensitivity analyses, adjustment for WC instead of BMI did not alter the associations in men and women (Table S5).

3.5 | Association of trajectories of glucose metabolism with hepatic iron and fat content

For men, trajectories of fasting insulin were tentatively associated with hepatic iron content (beta = 1.48, CI: [−0.02, 2.98]), while trajectories of 2-h glucose were significantly associated with hepatic iron content (beta = 1.27, CI: [0.12, 2.42], Table 3). Trajectories of fasting glucose, fasting insulin, HOMA-IR and 2-h glucose were significantly associated with hepatic fat content (Table 3), with similar effect sizes for fasting insulin, HOMA-IR and 2-h glucose (0.51, 0.51, 0.52 log(%), respectively). Cross-sectional 2-h insulin showed a significant association with hepatic fat content (0.31 log(%), CI: [0.20, 0.42]).

For women, trajectories of fasting glucose were associated with hepatic iron content when adjusted for age only, however, the association attenuated after further adjustment (Table 3). No further marker trajectories were associated with hepatic iron content. Trajectories of fasting glucose, fasting insulin and HOMA-IR were significantly associated with hepatic fat content (Table 3), with the strongest effect sizes for fasting insulin (0.63 log(%), CI: [0.36, 0.90]). Trajectories of 2-h glucose were only associated with hepatic fat content when adjusted for age, but the attenuated after further adjustment (0.21 log(%), CI: [−0.02, 0.44]). Cross-sectional 2-h insulin showed a significant association with hepatic fat content (0.33 log(%), CI: [0.21, 0.46]). Additional adjustment for menopausal status did not substantially affect the results (Table S4).

Results of two-step modelling remained similar after adjusting for WC instead of BMI in men and women (Table S6 and S7).

4 | DISCUSSION

We investigated longitudinal trajectories of a large panel of markers related to glucose metabolism, insulin resistance and diabetes along with MRI-derived parameters of hepatic iron and fat content in a sample from a population-based cohort. Our findings support a sex-specific association between dynamics of glucose metabolism and accumulation of fat and iron in the liver. Our main findings are threefold. First, trajectories of glycaemia were associated with hepatic iron and fat beyond cross-sectional associations. Second, associations between trajectories of markers of glucose metabolism and hepatic outcomes were stronger for hepatic fat than for hepatic iron content. Third, associations of continuous marker trajectories with hepatic fat content were stronger in women than in men.

Our findings are partly in line with a large population-based study on incident NAFLD diagnosed by abdominal ultrasound. Wang et al. found that changes in glycaemic traits conferred a higher risk for incident NAFLD than baseline values of glycaemic measures: For example, the risk ratio for the highest quartile of baseline 2-h glucose was 1.85 (CI: 1.43–2.40), whereas the risk ratio for changes from normal to impaired 2 h-glucose was 2.05 (1.71–2.45), and even higher for changes to the diabetic range.[23] Unfortunately, Wang et al. do not report sex-stratified effect estimates. A potential pathway linking glycaemia to increased hepatic fat content is oxidative stress induced by impaired hepatic mitochondrial capacity.[24] Moreover, in the large population-based SHIP study, Naeem et al. showed associations of MRI-derived hepatic iron content with 2-h glucose levels in participants without established T2D.[25] Our current results corroborate these findings and underscore the importance of taking dynamics of glucose metabolism into account for the risk estimation of excess hepatic fat content and iron.

In our sample, women had on average lower values of hepatic fat, less steatosis and less iron overload, which is in line with current knowledge about sex disparities in NAFLD prevalence.[7] However, we found stronger correlations of glucose markers with iron and fat content for women. Although NAFLD prevalence is lower in women, women are at a higher risk of progression to more severe disease states and advanced fibrosis,[26] which might be due to the loss of oestrogen and its anti-fibrotic effect during menopause. Furthermore, oestrogen promotes a favourable body composition pattern with a mainly gluteal and femoral distribution of adipose tissue. In contrast, the loss of oestrogen induces a shift to the accumulation of visceral adipose tissue, which is the major source of free fatty acids promoting NAFLD progression. We also note that in our analysis, effect estimates attenuated more strongly for women after adjustment for BMI. We have previously shown that hepatic iron content is associated with visceral fat in women but not in men.[21]

TABLE 3 Association of trajectories of markers of glucose metabolism with hepatic iron and fat.

			Men				Women			
			Hepatic iron content		Hepatic fat content		Hepatic iron content		Hepatic fat content	
Marker	Predictor	Adjustment	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Markers for the full sample										
HbA1c	Trajectory	Age	−0.08 (−1.10, 0.94)	.875	0.12 (−0.07, 0.31)	.202	0.52 (−0.55, 1.58)	.34	0.17 (−0.07, 0.40)	.158
	Value at Exam 1	Age	−0.88 (−1.92, 0.16)	.097	−0.03 (−0.22, 0.16)	.793	−0.51 (−1.51, 0.49)	.317	0.07 (−0.15, 0.30)	.506
HbA1c	Trajectory	Age, BMI, alcohol	0.08 (−0.93, 1.09)	.871	0.13 (−0.04, 0.29)	.131	0.49 (−0.54, 1.52)	.348	0.11 (−0.10, 0.32)	.291
	Value at Exam 1	Age, BMI, alcohol	−1.01 (−2.04, 0.02)	.054	−0.08 (−0.25, 0.09)	.357	−0.54 (−1.51, 0.42)	.269	0.04 (−0.15, 0.24)	.669
Fasting glucose	Trajectory	Age	0.48 (−0.42, 1.38)	.292	0.21 (0.05, 0.37)	.009	1.18 (0.24, 2.13)	**.014**	0.39 (0.20, 0.58)	<.001
	Value at Exam 1	Age	−0.85 (−1.77, 0.07)	.07	0.01 (−0.15, 0.17)	.894	−0.48 (−1.43, 0.46)	.317	0.02 (−0.17, 0.22)	.798
Fasting glucose	Trajectory	Age, BMI, alcohol	0.47 (−0.41, 1.36)	.291	0.17 (0.03, 0.31)	.016	0.8 (−0.17, 1.76)	.106	0.26 (0.08, 0.44)	.006
	Value at Exam 1	Age, BMI, alcohol	−0.94 (−1.85, −0.03)	.043	−0.03 (−0.17, 0.12)	.709	−0.33 (−1.26, 0.60)	.486	0.05 (−0.13, 0.22)	.6
Fasting insulin	Trajectory	Age	1.43 (−0.08, 2.94)	.063	0.54 (0.31, 0.77)	<.001	1.41 (−0.11, 2.93)	.069	0.7 (0.45, 0.96)	<.001
	Value at Exam 1	Age	−1.70 (−3.20, −0.19)	.028	−0.09 (−0.32, 0.14)	.456	−0.92 (−2.42, 0.59)	.231	−0.19 (−0.45, 0.07)	.144
Fasting insulin	Trajectory	Age, BMI, alcohol	1.48 (−0.02, 2.98)	.053	0.51 (0.29, 0.73)	<.001	0.9 (−0.66, 2.46)	.256	0.63 (0.36, 0.90)	<.001
	Value at Exam 1	Age, BMI, alcohol	−1.80 (−3.34, −0.27)	.021	−0.17 (−0.39, 0.06)	.152	−0.68 (−2.17, 0.80)	.366	−0.18 (−0.44, 0.07)	.162
Markers for the sample without previously established diabetes/without glucose-lowering medication										
HOMA-IR	Trajectory	Age	1.12 (−0.23, 2.48)	.104	0.54 (0.33, 0.75)	<.001	1.25 (−0.13, 2.63)	.076	0.69 (0.46, 0.92)	<.001
	Value at Exam 1	Age	−0.83 (−2.18, 0.52)	.226	−0.07 (−0.28, 0.14)	.507	−0.76 (−2.12, 0.61)	.275	−0.22 (−0.44, 0.01)	.058
HOMA-IR	Trajectory	Age, bmi, alcohol	1.16 (−0.19, 2.52)	.091	0.51 (0.31, 0.71)	<.001	0.7 (−0.74, 2.13)	.338	0.60 (0.36, 0.85)	<.001
	Value at Exam 1	Age, BMI, alcohol	−1.12 (−2.49, 0.25)	.11	−0.18 (−0.38, 0.03)	.096	−0.48 (−1.83, 0.87)	.485	−0.22 (−0.45, 0.01)	.06
2-h glucose	Trajectory	Age	1.14 (−0.01, 2.29)	.051	0.58 (0.38, 0.78)	<.001	0.67 (−0.58, 1.92)	.29	0.36 (0.13, 0.59)	.003
	Value at Exam 1	Age	0.04 (−1.12, 1.20)	.945	−0.21 (−0.41, −0.01)	.036	−0.59 (−1.83, 0.66)	.352	0.03 (−0.20, 0.27)	.793
2-h glucose	Trajectory	Age, bmi, alcohol	1.27 (0.12, 2.42)	.03	0.52 (0.35, 0.70)	<.001	0.37 (−0.89, 1.63)	.564	0.21 (−0.02, 0.44)	.074
	Value at Exam 1	Age, BMI, alcohol	−0.22 (−1.37, 0.93)	.708	−0.28 (−0.46, −0.10)	.002	−0.52 (−1.72, 0.67)	.391	0.05 (−0.17, 0.27)	.637
2-h insulin	Value at Exam 2	Age	0.37 (−0.30, 1.04)	.278	0.41 (0.30, 0.52)	<.001	0.15 (−0.47, 0.76)	.642	0.43 (0.32, 0.54)	<.001
2-h insulin	Value at Exam 2	Age, BMI, alcohol	0.29 (−0.41, 0.99)	.412	0.31 (0.20, 0.42)	<.001	−0.13 (−0.83, 0.57)	.713	0.33 (0.21, 0.46)	<.001

Note: Results from two-step multi-level modelling, with trajectories derived from a linear mixed-model with random slopes, adjusted for changes in glucose-lowering medication. The beta coefficient for trajectories thus denotes effects of the standardized individual deviation from population change. Hepatic fat content was log-transformed before analysis.

Data from the current analysis showed a stronger correlation of BMI with liver iron in women ($r=0.25$) than in men ($r=0$), indicating body composition pattern and hepatic iron content are more closely connected in women. Interestingly, we observed that HOMAR-IR and fasting insulin decreased in women with diabetes, which we did not observe in men. This could be explained by the increased number of postmenopausal women and the increased number of glucose-lowering medications.

We found no association of trajectories of HbA1c with hepatic iron or fat content. Hb1Ac is a glycosylated protein stemming from the interaction of glucose and haemoglobin and is a measure of average blood glucose levels in the preceding 2–3 months. Wang et al. reported changes in HbA1c levels to be associated with incident NAFLD,[23] whereas a recent study using cross-sectional NHANES data reported an association of HbA1c measurements with prevalent NALFD in individuals without diabetes only in participants with BMI ≥30 kg/m^2.[27] This again indicates a possible modulating role of body composition in the association of HbA1c and hepatic phenotypes.

In women, diabetes confers a higher risk for cardiovascular diseases (CVD) and CVD mortality compared to men.[28] This excess risk cannot be explained by underlying traditional confounders[28] and cannot be captured by HbA1c measurements alone.[29] In our analysis, we found a similar pattern in the association of diabetes-related markers and hepatic phenotypes, so one could hypothesize that hepatic steatosis and iron overload might modulate the excess CVD risk in women with diabetes. Excess hepatic lipogenesis leads to secretion of very low-density lipoproteins that induce triglyceride accumulation in other peripheral tissues. Moreover, increased hepatic triglyceride accumulation is accompanied by chronic inflammation, promoting vascular inflammation and vasoconstriction. Current evidence on the independent association of NAFLD with CVD beyond shared risk factors is inconclusive,[30] but a recent study by Pafili et al found a potential mediator role of visceral adipose tissue in the development of NAFLD.[31] In obese patients with NAFLD, mitochondrial respiration in visceral adipose tissue was downregulated compared with obese subjects without NAFLD. In addition, they observed a link of lower insulin sensitivity in adipose tissue and impaired VAT respiration.[31] Further studies are needed that analyse the potentially mediating effect of hepatic phenotypes on the association of diabetes with CVD in women.

The causal, bi-directional, association between diabetes and NAFLD has already been established.[5] Since imaging data was only available at the last examination time point in our study, we cannot derive the temporal sequence of deterioration of glycaemia and accumulation of hepatic iron and fat. We observed substantially higher values of hepatic fat in men who progressed to diabetes compared to those with sustained diabetes, indicating that the dynamics of glucose metabolism might exacerbate existing or induce new accumulation of hepatic adipose tissue. However, it would also be possible that individuals with prevalent diabetes already underwent treatment, counselling, or have implemented lifestyle changes to monitor their risk factors more closely, which would lead to decreased NAFLD risk. Moreover, for men, incident prediabetes was associated with higher hepatic iron content whereas for women, there was no clear association of deteriorating glycaemia with hepatic iron content. There was no significant association of any marker trajectory with hepatic iron content in women. This again underlines that in women factors like body composition and menopausal status might influence the association between diabetes and iron metabolism to a different extent than in men. Regarding causality, there are currently no Mendelian randomization studies on the causal relation between hepatic iron content and glycaemia.

Strengths of our study include the use of a well-characterized sample from a population-based study with a large panel of glucose metabolism markers available. Exposure and outcome data were derived by OGTT and MRI, respectively, which are considered gold standards for these measures. Within the longitudinal setup, the availability of two time points allowed us to investigate incident diabetes and prediabetes as well as marker trajectories. However, our study also has limitations. Most prominently, the MRI-derived outcomes were only available at one time point which prevented us from establishing bi-directional longitudinal associations. Moreover, sample sizes are insufficient for more complex analyses, including further stratification, e.g. according to genotype at relevant SNPs or menopausal status.

5 | CONCLUSION

In conclusion, unfavourable longitudinal trajectories of markers of glucose metabolism are associated with increased hepatic fat content. Associations with hepatic iron content are more complex and deserve further investigation regarding the role of body composition and menopause.

Monitoring trajectories of glycaemia in the sub-diabetic range, particularly in women, might enable the early detection of unfavourable liver phenotypes and vice versa.

AUTHOR CONTRIBUTIONS

Conceptualization: Susanne Rospleszcz; Methodology: Fiona Niedermayer, Susanne Rospleszcz; Formal analysis: Fiona Niedermayer, Yaqi Su, Susanne Rospleszcz; Resources: Ricarda von Krüchten, Barbara Thorand, Annette Peters, Wolfgang Rathmann, Michael Roden, Christopher L. Schlett, Fabian Bamberg, Johanna Nattenmüller; Data Curation: Fiona Niedermayer, Yaqi Su, Ricarda von Krüchten, Barbara Thorand, Annette Peters, Wolfgang Rathmann, Michael Roden, Christopher L. Schlett, Fabian Bamberg, Johanna Nattenmüller, Susanne Rospleszcz; Writing—Original Draft: Fiona Niedermayer, Yaqi Su, Susanne Rospleszcz; Writing—Review & Editing: Fiona Niedermayer, Yaqi Su, Ricarda von Krüchten, Barbara Thorand, Annette Peters, Wolfgang Rathmann, Michael Roden, Christopher L. Schlett, Fabian Bamberg, Johanna Nattenmüller, Susanne Rospleszcz; Visualization: Fiona Niedermayer; Supervision: Susanne Rospleszcz; Funding acquisition: Barbara Thorand, Annette

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The informed consent given by KORA study participants does not cover data posting in public databases. However, data are available upon request by means of a project agreement. Requests should be sent to kora.passt@helmholtz-muenchen.de and are subject to approval by the KORA Board.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the ethics committee of Ludwig-Maximilians-University Munich (498–12) and the Bavarian Chamber of Physicians (FF4: EC No. 06068) and was performed according to the Declaration of Helsinki.

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